Helicobacter pylori-specific Protection Against Inflammatory Bowel Disease Requires the NLRP3 Inflammasome and IL-18

Engler, Daniela B ; Leonardi, Irina ; Hartung, Mara L ; Kyburz, Andreas ; Spath, Sabine ; Becher, Burkhard ; Rogler, Gerhard ; Müller, Anne

Abstract: BACKGROUND The Gram-negative bacterium Helicobacter pylori is a constituent of the human gastric microbiota. Chronic infection with H. pylori causes gastritis and predisposes to gastric carcinoma but has also been inversely linked to various allergic and chronic inflammatory conditions. In particular, large meta-analyses have documented an inverse association between H. pylori infection and the risk of developing ulcerative colitis and Crohn's disease. METHODS We investigated possible protective effects of experimental H. pylori infection and of regular treatment with H. pylori extract in 2 mouse models of colitis and in mouse models of type I diabetes and multiple sclerosis. The mechanism of protection was examined in mouse strains lacking specific innate immune recognition pathways and cytokines. RESULTS We show here that experimental infection with H. pylori and administration of regular doses of H. pylori extract both alleviate the clinical and histopathological features of dextran sodium sulfate-induced chronic colitis and of T-cell transfer-induced colitis. High resolution endoscopy of the protected animals revealed the accumulation of large amounts of colonic mucus upon H. pylori exposure, which could be attributed to transcriptional activation of the mucin 2 gene. The protection against dextran sodium sulfate-induced colitis was dependent on the NLRP3 inflammasome and interleukin-18 signaling. Other autoimmune diseases, i.e., experimental autoimmune encephalomyelitis and type I diabetes, were not controlled by H. pylori. CONCLUSIONS In summary, we propose here that the immunomodulatory activity of an ancient constituent of the gut microbiota, H. pylori, may be exploited for the prevention and/or treatment of inflammatory bowel diseases.

DOI: https://doi.org/10.1097/MIB.0000000000000318

Helicobacter pylori–specific Protection Against Inflammatory Bowel Disease Requires the NLRP3 Inflammasome and IL-18

Daniela B. Engler, PhD, Irina Leonardi, MSc,† Mara L. Hartung, Msc,* Andreas Kyburz, MSc,* Sabine Spath, PhD,‡ Burkhard Becher, PhD,‡ Gerhard Rogler, MD, PhD,† and Anne Müller, PhD**

Background: The Gram-negative bacterium *Helicobacter pylori* is a constituent of the human gastric microbiota. Chronic infection with *H. pylori* causes gastritis and predisposes to gastric carcinoma but has also been inversely linked to various allergic and chronic inflammatory conditions. In particular, large meta-analyses have documented an inverse association between *H. pylori* infection and the risk of developing ulcerative colitis and Crohn's disease.

Methods: We investigated possible protective effects of experimental *H. pylori* infection and of regular treatment with *H. pylori* extract in 2 mouse models of colitis and in mouse models of type I diabetes and multiple sclerosis. The mechanism of protection was examined in mouse strains lacking specific innate immune recognition pathways and cytokines.

Results: We show here that experimental infection with *H. pylori* and administration of regular doses of *H. pylori* extract both alleviate the clinical and histopathological features of dextran sodium sulfate–induced chronic colitis and of T-cell transfer-induced colitis. High resolution endoscopy of the protected animals revealed the accumulation of large amounts of colonic mucus upon *H. pylori* exposure, which could be attributed to transcriptional activation of the mucin 2 gene. The protection against dextran sodium sulfate–induced colitis was dependent on the NLRP3 inflammasome and interleukin-18 signaling. Other autoimmune diseases, i.e., experimental autoimmune encephalomyelitis and type I diabetes, were not controlled by *H. pylori*.

Conclusions: In summary, we propose here that the immunomodulatory activity of an ancient constituent of the gut microbiota, *H. pylori,* may be exploited for the prevention and/or treatment of inflammatory bowel diseases.

(Inflamm Bowel Dis 2015;0:1–8)

Key Words: chronic intestinal inflammation, microbial immunomodulation, autoimmunity, mucus production, inflammasome activation

Crohn's disease (CD) and ulcerative colitis (UC), collectively referred to as inflammatory bowel diseases (IBDs), develop in genetically susceptible individuals as the result of an inappropriately aggressive immune response to ubiquitous antigens of the normal intestinal microflora. Genetic studies have highlighted the importance of host–microbe interactions and of innate immune recognition of components of the intestinal microbiota in the pathogenesis of these chronic inflammatory disorders.[1] The prevalence of both IBDs has increased in most developed countries during the past century, a trend that has been attributed to changes in diet, antibiotic use, and environmental factors and to changing patterns of intestinal colonization.[2] The biological hallmarks of active inflammatory bowel disease are a pronounced infiltration of innate and adaptive immune cells into the lamina propria and elevated local levels of their cytokine products, especially tumor necrosis factor-α, IL-1β, interferon (IFN)-γ, and Th17 cell-derived cytokines. Approved treatments for IBDs include aminosalicylates or topical corticosteroids for mild disease forms and systemic corticosteroids, immunosuppressive thiopurines, and biologics targeting tumor necrosis factor-α for moderately active or severe disease courses or acute flares.[3] Alternative therapeutic strategies in (pre) clinical development target cytokines involved in IBD pathogenesis (IL-12 and IL-23), the α4β7 integrin involved in lymphocyte homing to the gut, and JAK3 kinase, which is involved in cytokine signaling.[4] Additionally, rather experimental strategies attempt to modulate pathogenic immune responses in models of IBD by the introduction of helminths, such as *Heligmosomoides polygyrus*[5,6] or *Trichuris suis*[7,8] or of probiotics (especially Lactobacillus or Bifidobacterium species or the Escherichia coli strain Nissle).[9]

Chronic gastric infection with the bacterial pathogen *Helicobacter pylori* causes gastritis and peptic ulcer disease[10] and represents the most important risk factor for gastric cancer[11,12] but has also been linked inversely to the risk of developing allergic diseases and IBD in large epidemiological studies and

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.ibdjournal.org).

Received for publication November 18, 2014; Accepted November 18, 2014.

From the *Institutes of Molecular Cancer Research, University of Zürich, Zürich, Switzerland; ‡Experimental Immunology, University of Zürich, Zürich, Switzerland; and †Division of Gastroenterology and Hepatology, University Hospital of Zürich, Zürich, Switzerland.

Supported by the Gebert Rüf Foundation (Grant number GRS-043/11 to A.M.).

The authors have no conflicts of interest to disclose.

Reprints: Anne Müller, PhD, Institute of Molecular Cancer Research, University of Zürich, Winterthurerstr 190, 8057 Zürich, Switzerland (e-mail: mueller@imcr.uzh.ch).

DOI 10.1097/MIB.0000000000000318

Published online.

meta-analyses.[13–15] We have shown in various models that experimental *H. pylori* infection prevents allergen-induced asthma by inducing tolerogenic DCs, which acquire the ability to direct the differentiation of protective FoxP3$^+$ regulatory T cells.[16–19] We report here that experimental *H. pylori* infection and regular administration of *H. pylori* extract protect against chronic colitis in the dextran sodium sulfate (DSS) and T-cell transfer models of the disease. Protective effects were evident during colonoscopy and upon histological assessment of affected colonic tissues. Efficient protection from the chronic inflammation typical of DSS-induced colitis required innate immune recognition of *H. pylori* by the NLRP3 inflammasome and the subsequent production and secretion of IL-18. NLRP3$^{-/-}$ as well as IL-18$^{-/-}$ and IL-18R$^{-/-}$ mice or mice lacking the downstream adaptor protein MyD88, failed to respond to *H. pylori* extract treatment. The endoscopy approach further revealed that *H. pylori* extract induced the production of large quantities of protective mucus composed of the intestinal mucin 2 (MUC2). Other autoimmune diseases, such as type I diabetes (T1D), and experimental autoimmune encephalomyelitis were not controlled by *H. pylori* infection or *H. pylori* extract administration. In summary, we provide evidence for the efficacy of a new treatment strategy for IBD that exploits the immunomodulatory properties of *H. pylori*, a naturally occurring infection known to be inversely correlated with IBD risk in humans.

MATERIALS AND METHODS

Animal Experimentation

C57BL/6, BL/6.Nlrp3$^{-/-}$, BL/6.MyD88$^{-/-}$, BL/6.IL18$^{-/-}$, and BL/6.IL18R$^{-/-}$ mice were obtained from Jackson Laboratories and maintained in individually ventilated cages under SPF conditions. Eight-week-old mice were subjected to 3 cycles of 5 days each of 2% DSS followed by 1 week-long compound-free intervals. To induce colitis in T-cell–deficient hosts, BL/6.TCR$\beta^{-/-}$ mice (Jackson Labs) were intraperitoneally (i.p.) injected with 400,000 naive CD4$^+$CD62L$^+$CD44^{lo} T cells (purified by immunomagnetic isolation, MagCellect Naive T cell kit; R&D). Mice were either orally infected at 7 days of age with 10^8 *H. pylori* PMSS1 as described[20] or received 3 weekly oral or i.p. doses of 200 μg extract of *H. pylori* PMSS1 beginning after the first cycle of DSS treatment or on the day of T-cell transfer. Colon length was measured from the cecum to the distal rectum; colons were dissected longitudinally with one half being embedded for formalin fixation and paraffin embedding and the other being cryopreserved for later RNA extraction. Paraffin sections were stained with Giemsa and examined in blinded fashion on a BX40 Olympus microscope. For induction of experimental autoimmune encephalomyelitis (EAE), C57BL/6 mice were immunized with 200 μg myelin oligodendrocyte glycoprotein fragment 35-55 (MOG_{35-55}) peptide adjuvanted by complete Freund's adjuvant, followed by two 200 ng i.p. injections of pertussis toxin. Spontaneously developing type 1 diabetes was assessed in female nonobese diabetic mice (NOD/ShiLtJ mice, Jackson labs) at 20 weeks of age. For induction of T1D, male NOD mice were i.p. injected with one 5 mg dose of cyclophosphamide at 10 weeks of age and assessed at 20 weeks of age. The pancreas was formalin-fixed and paraffin-embedded, and H&E-stained sections were scored as described below. The inflammation of 30 pancreatic islets was recorded per animal. All animal experimentation was performed in accordance with federal, cantonal, and institutional guidelines and was approved by the Zurich Cantonal Veterinary Authorities.

Histopathological Analysis of Colitis, Insulitis, and Gastritis, and Clinical Scoring of Colitis and EAE

The scoring system first introduced by Asseman et al[21] was used for the quantitative histopathological assessment of colitis. A grade of 0 was given when no changes were observed; grade 1, minimal scattered mucosal inflammatory cell infiltrates, with or without minimal epithelial hyperplasia; grade 2, mild scattered to diffuse inflammatory cell infiltrates, sometimes extending into the submucosa and associated with erosions, with minimal to mild epithelial hyperplasia and minimal to mild mucin depletion from goblet cells; grade 3, mild-to-moderate inflammatory cell infiltrates that were sometimes transmural, often associated with ulceration, with moderate epithelial hyperplasia and mucin depletion; grade 4, marked inflammatory cell infiltrates that were often transmural and associated with ulceration with marked epithelial hyperplasia and mucin depletion; grade 5, marked transmural inflammation with severe ulceration. Colitis endoscopy scores were defined for the following 5 parameters[22]: thickening of the colon: 0, transparent; 1, moderate; 2, marked, 3, nontransparent; changes of the vascular pattern: 0, normal; 1, moderate; 2, marked; 3, bleeding; fibrin visible: 0, none; 1, little; 2, marked; 3, extreme; granularity of the mucosal surface: 0, none; 1, moderate; 2, marked; 3, extreme; stool consistency: 0, normal and solid; 1, still shaped; 2, unshaped; 3, spread. Overall scores were determined by adding all individual scores to reach 0 to 15. For the daily clinical scoring of EAE, we used a previously published procedure[23] as follows: 0, no detectable signs of EAE; 0.5, distal limp tail; 1, completely limp tail; 1.5, limp tail and hind limb weakness; 2, unilateral partial hind limb paralysis; 2.5, bilateral partial hind limb paralysis; 3, complete bilateral hind limb paralysis; 3.5, complete bilateral hind limb paralysis and partial forelimb paralysis; 4, moribund (mouse completely paralyzed); 5, dead. Food and water were provided in the cage after scores of >1 were reached. H&E-stained sections of pancreas were scored for insulitis based on a previously published system.[24] Four grades of insulitis were distinguished: grade 0, absence of insulitis; 1, peri-insulitis; 2, light infiltration involving $<25\%$ of the islet area; 3, heavy infiltration involving $>25\%$ of the islet area. The degree of *H. pylori*–induced gastritis/inflammation, atrophy, and hyperplasia was scored on H&E-stained gastric sections based on the modified "Sydney system," as published previously.[25] Briefly, scores for chronic inflammation were 0, none; 1, some infiltrates; 2, mild (few aggregates in submucosa and

mucosa); 3, moderate (several aggregates in submucosa and mucosa); 4, marked (many big aggregates in submucosa and mucosa); 5, nearly the entire mucosa contains a dense infiltrate; and 6, entire mucosa contains a dense infiltrate. Scores for atrophy were 0, none; 1, foci, where a few gastric glands are lost or replaced; 2, small areas in which gastric glands have disappeared or been replaced; 3, 25% of gastric glands lost or replaced; 4, 25% to 50% of gastric glands lost or replaced; 5, 50% of gastric glands lost or replaced; 6, only a few small areas of gastric differentiated glands remaining. For hyperplasia, the scores were 0, none; 1, single glands (next to infiltrate); 2, 1 focal area/1 to 4 crypts (mild); 3, 1 to 3 foci; 4, multiple foci; 5, 50% of glands affected; 6, only few small nonhyperplasic areas.

Preparation of *H. pylori* Extract and Quantification of *H. pylori* Colonization by Colony Count Assay

Helicobacter pylori was cultured in Brucella broth supplemented with 10% fetal calf serum, pelleted by centrifugation and washed once with phosphate-buffered saline. Bacteria were subjected to 3 freeze/thaw cycles and disrupted by 3 passes through a French pressure cell press (Stansted Fluid Power, Cell Pressure Homogenizer) at 30.000 bar. Cell debris was removed by centrifugation and the supernatant was filtered through a 2-μm filter. Protein concentrations were determined by BCA Protein Kit (R&D systems). For the quantitative assessment of *H. pylori* colonization, one section of each stomach was transferred to a tube containing Brucella broth and homogenized with an Ultra Turrax homogenizer (John Morris Scientific Ltd., Chatswood, Australia). Serial dilutions were plated on horse blood plates to determine bacterial loads.

RNA Extraction, Reverse Transcription, and Quantitative PCR

For real-time RT-PCR, total RNA was isolated from 1 longitudinal section of the colon using NucleoSpin RNA II kits (Macherey-Nagel, Düren, Germany) and reversely transcribed using SuperScript III Reverse Transcriptase (Life Technologies, Carlsbad, CA). The corresponding cDNA served as a template for real-time PCR performed using the LightCycler 480 SYBR Green I master kit (Roche, Basel, Switzerland). Absolute values of MUC2, CDX-2, TGF-β, IL-17, and IFN-γ expression were normalized to GAPDH expression (conditions: 60°C annealing temperature, 50 cycles). The following primers were used: GAPDH, forward: 5′-GAC ATT GTT GCC ATC AAC GAC C-3′, reverse: 5′-CCC GTT GAT GAC CAG CTT CC-3′; MUC2, forward: 5′-TGCCCAGAGAG TTTGGAGAG-3′, reverse: 5′-CCTCACATGTGGTCTGGTTG-3′; CDX-2, forward: 5′-CTGGGGTTCTGAAACCAAAT-3′, reverse: 5′-CACCATCAGGAGGAAAAGTGA-3′; TGFb, forward: 5′-TG ACGTCACTGGAGTTGTACGG-3′, reverse: 5′-GGTTCATG TCATGGATGGTGC-3′; IFNg, forward: 5′-CATGGCTGTTT CTGGCTGTTACTG-3′; reverse: 5′-GTTGCTGATGGCCTG ATTGTCTTT-3′, IL-17, forward: 5′-GCTCCAGAAGGCCCT CAG A-3′; reverse: 5′-AGCTTTCCCTCCGCATTGA-3.′

Statistics

All statistical analyses were performed using Graph Pad prism 5.0 software (Graph Pad Software, San Diego, CA). The Mann–Whitney test was used for all comparisons except for those in EAE, where 2-way analysis of variance (ANOVA) was used to determine significant differences over time. P values <0.05 were considered significant.

RESULTS

Live *H. pylori* and *H. pylori* Extract Protect Effectively Against the Clinical and Histopathological Features of Chronic Colitis

To assess whether experimental infection with *H. pylori* or regular administration of *H. pylori* extract modulate the severity of DSS-induced colitis, C57BL/6 mice were either experimentally infected with 1 orogastric dose of the mouse-colonizing *H. pylori* strain PMSS1 at 7 days of age or received intragastrically administered *H. pylori* extract (10 mg/kg body weight) 3 times a week. All mice were exposed to three 5-day long cycles of DSS in the drinking water (2% final concentration), each followed by a compound-free interval of 1 week. At the study endpoint, mice were examined by high resolution colonoscopy and assessed with respect to colonic histopathology and colon length. Histopathological evaluation of the colon revealed that all mice of the positive control group had developed moderate-to-severe colitis, as evidenced by the widespread loss of goblet cells and of crypts, accompanied by extensive mucosal and submucosal infiltration of inflammatory cells (Fig. 1A, B). In contrast, *H. pylori*–infected mice exhibited significantly less inflammation and had also undergone less substantial epithelial changes (Fig. 1A, B). Interestingly, a similar beneficial effect was observed in mice that were treated with *H. pylori* extract. Overall, significantly lower histopathology scores were assigned to the mice in the 2 treatment arms exposed to live *H. pylori* or its extract than to the positive controls (Fig. 1A, B). Lower histopathology scores were associated with a decreased expression of the Th1 and Th17 cytokines IFN-γ and IL-17 in the colonic mucosa of the infected and treated mice (see Fig. A and B, Supplemental Digital Content 1, http://links.lww.com/IBD/A714). An additional readout of colitis pathology known to correlate well with the histopathological gold standard is colon length. Mice in the positive control group exhibited substantially shorter colons than negative controls that had never been exposed to DSS; *H. pylori* infection or extract treatment restored normal colon length (Fig. 1C). Finally, high resolution endoscopy[22] was used to quantitatively assess colitis in a subset of the mice shown in Figure 1A. Whereas DSS-treated mice of the positive control group were characterized by thickening and intransparency of the colon, mucosal bleeding, abundant fibrin, granularity of the mucosal surface, and loose stools, all of these parameters were less severe in the *H. pylori*–infected or extract-treated mice (Fig. 1D, E). Consequently, the overall colonoscopy scores assigned on a scale from 0 to 15[22] for

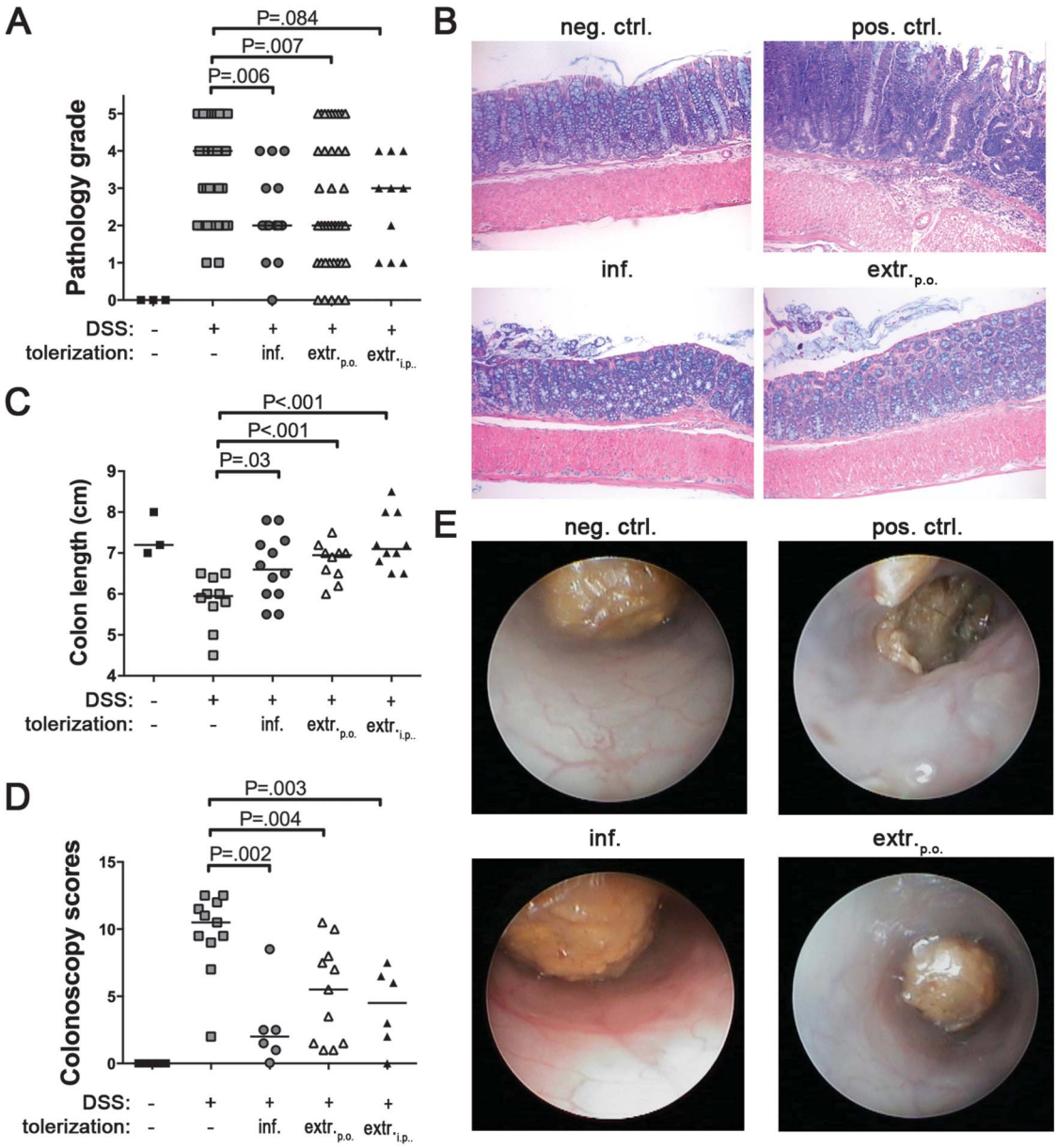

FIGURE 1. *Helicobacter pylori* infection and *H. pylori* extract treatment protect against DSS-induced colitis. C57BL/6 mice were either infected with 1 orogastric dose of live *H. pylori* (strain PMSS1) at 7 days of age or received 3 weekly doses of 200 μg *H. pylori* extract from the day of the first DSS cycle onwards, administered either i.p. or perorally (p.o.) and were subjected to three 5-day cycles of 2% DSS in the drinking water. A and B, Histopathology scores and representative micrographs of Giemsa-stained tissue sections. Pooled data from 2 (inf., extr.$_{i.p.}$) to 5 (pos. ctrl., extr.$_{p.o.}$) independent studies are shown. C, Colon lengths as determined from cecum to distal rectum for a subset of the mice shown in (A). D and E, Colonoscopy scores and representative endoscopic images of a subset of the mice shown in (A). A, C, and D, Each data point represents 1 mouse; horizontal lines indicate the medians. INF, infected; EXTR, extract-treated; POS CTRL, positive control; and NEG CTRL, negative control.

the above-mentioned parameters were reduced from an average of 10 (positive controls) to 5 (infected and extract treated; Fig. 1D). Interestingly, lower doses of extract (<10 mg/kg body weight) or once-weekly treatment with the 10 mg/kg dose were not sufficient to confer protection (data not shown), whereas the i.p. injection of *H. pylori* extract conferred a level of protection that was quite similar to the protection provided by orogastric delivery (Fig. 1A–E).

To determine whether the observed protective effects are specific to the DSS model of barrier disruption followed by microbiota-induced autoinflammation, we assessed the efficacy of extract treatment in the T-cell transfer-mediated model of colitis.[26] Lymphopenic recipients of adoptively transferred naive $CD4^+$ T cells were either subjected to 3 times weekly treatment with *H. pylori* extract or remained untreated. Whereas the recipients of naive T cells in the positive control group developed severe

colitis as determined by loss of body weight as well as histopathological and endoscopical analysis, all symptoms were strongly reduced in extract-treated animals (see Fig. C–E, Supplemental Digital Content 1, http://links.lww.com/IBD/A714). All measured parameters are thus consistent with protective effects of live *H. pylori* infection and/or extract treatment on the development of chronic colitis in 2 models of IBD. No adverse effects of the extract treatment could be detected on the gastric mucosa of a non-colitic control group (see Fig. F–G, Supplemental Digital Content 1, http://links.lww.com/IBD/A714), neither in the presence or absence of an experimental *H. pylori* infection, indicating that the treatment does not induce or aggravate *H. pylori*–associated gastric pathology.

Helicobacter pylori Infection or Extract Treatment Do not Ameliorate Experimental Autoimmune Encephalomyelitis or Type 1 Diabetes

Hypothesizing that other autoinflammatory or autoimmune conditions might also be prevented or ameliorated by *H. pylori*, we examined the effects of *H. pylori* infection or extract treatment in models of multiple sclerosis and T1D. To assess the effects of *H. pylori* on multiple sclerosis–like autoimmune neuroinflammation, we immunized wild-type C57BL/6 mice with MOG_{35-55} peptide to trigger EAE. All mice developed central nervous system inflammation as determined by scoring of the progressive paralysis of tail and hind limbs within ~9 to 13 days postimmunization; *H. pylori* infection or 3 times weekly treatment with *H. pylori* extract did not measurably affect the course of disease (Fig. 2A, B); if anything, extract treatment made the condition worse. Similarly, *H. pylori* infection of female NOD mice, which spontaneously develop pancreatic islet inflammation and represent a widely used model of T1D, did not result in improved pancreatic histopathology (Fig. 2C, D). Finally, in a parallel model of inducible T1D, male NOD mice were subjected to a single dose of cyclophosphamide to trigger pancreatic islet inflammation; as in the spontaneous model, neither live infection nor extract treatment alleviated disease symptoms (Fig. 2E), suggesting that T1D develops regardless of the presence of *H. pylori*. We verified that *H. pylori* is fully competent to colonize NOD mice, albeit at lower levels than the C57BL/6 mice used in the DSS-induced colitis model (Fig. 2F). In conclusion, our results indicate that prototypical autoimmune diseases are not controlled by live *H. pylori* and therefore also fail to respond to *H. pylori* extract treatment.

Protection Against Colitis Conferred by Live *H. pylori* or Its Extract Is Accompanied by MUC2 Upregulation and Depends on the NLRP3 Inflammasome and IL-18 Signaling

The most striking endoscopic feature of mice that are protected against experimentally induced colitis by either live *H. pylori* infection or extract treatment is their abundant mucus production (Fig. 1E). The predominant intestinal mucin, MUC2, is produced by goblet cells and forms an insoluble barrier that protects the intestinal epithelium against colonization by gut microbes.[27] We investigated the expression of MUC2 transcript and, in accordance with the endoscopy results, found it to be strongly upregulated in the intestinal mucosa of *H. pylori*–infected and extract-treated mice relative to colitic controls (Fig. 3A). The intestinal transcription factor CDX2, which regulates MUC2 production,[28] is upregulated in a similar manner in the protected mice (Fig. 3B). Having identified a copy of the SMAD binding motif CAGACA[29] in the CDX2 promoter sequence, we speculated that the regulatory cytokine TGF-β, known to be critically involved in *H. pylori*–specific immunomodulation and in intestinal homeostasis,[20,30] might play a role in *H. pylori*–induced colitis protection. The expression pattern of TGF-β indeed paralleled those of MUC2 and CDX2 (Fig. 3C), suggesting that the TGF-β-CDX2-MUC2 axis represents a strong correlation of, or is functionally involved in, colitis protection. To examine whether MUC2 induction by *H. pylori* extract is a phenomenon linked to DSS treatment, we treated a large group of mice with 3 times weekly doses of *H. pylori* extract without exposing them to DSS at any time; this treatment recapitulated the strong MUC2 induction seen in DSS-treated mice (Fig. 3D), indicating that intestinal MUC2 upregulation is a direct result of the exposure to *H. pylori* extract rather than a consequence of the protection from DSS colitis.

We and others have reported recently that *H. pylori* activates the inflammasome and caspase-1 to induce the processing and secretion of IL-1β and IL-18.[31,32] IL-18 signaling is required for the protection against allergic asthma that is conferred by live infection[17] and *H. pylori* extract treatment.[18] Several polymorphisms in the IL-18 gene are known to contribute to UC susceptibility.[33] To test whether a functional NLRP3 inflammasome, the predominant inflammasome detecting *H. pylori*,[32] and IL-18 signaling proficiency are required for *H. pylori* extract-mediated protection, we subjected mice lacking NLRP3, IL-18, IL-18 receptor, or the adaptor protein MyD88 to DSS-induced colitis and treated the mice with *H. pylori* extract. None of these strains were protected against DSS-induced colitis, despite the fact that wild-type mice examined in the same experiment exhibited a typical level of protection (Fig. 3E). The activation of the NLRP3 inflammasome and subsequent secretion of IL-18 upon extract treatment thus are important prerequisites of *H. pylori* extract–mediated colitis protection.

DISCUSSION

The combined results presented here demonstrate that *H. pylori*, administered either live or in extract form, confers protection against IBD in standard mouse models of the disease. Our data are consistent with a strong inverse correlation of *H. pylori* seropositivity with the risk of developing both CD and UC,[13,15] as well as with experimental data documenting a protective effect of live *H. pylori* infection on acute *Salmonella typhimurium*–induced

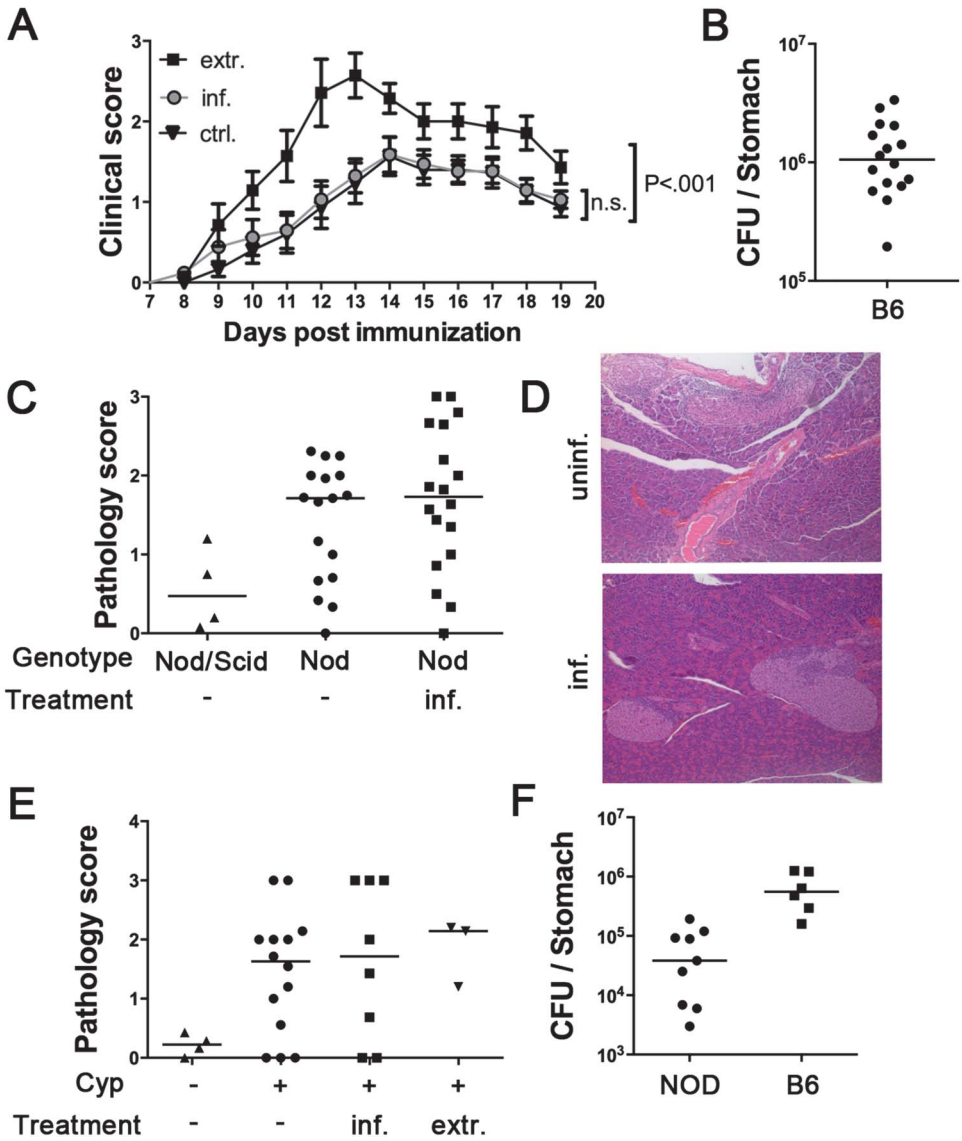

FIGURE 2. *Helicobacter pylori* infection and *H. pylori* extract treatment fail to protect against EAE and T1D. A and B, C57BL/6 mice were either infected on day 7 of age with *H. pylori* (16 mice) or received 3 weekly i.p. doses of 200 μg *H. pylori* extract from day 7 of age onwards (12 mice), or remained untreated (24 mice). All mice were immunized with MOG_{35-55} peptide at 6 weeks of age and monitored for EAE symptoms for 19 days thereafter. Averages ± SDs of EAE scores of the 3 treatment groups are shown in (A). Colony-forming units per stomach as determined by plating and colony counting are shown in (B) for all infected mice. Pooled data from 2 independent studies are shown in (A) and (B). C and D, Female NOD mice were either infected on day 7 of age with *H. pylori* (18 animals) or remained uninfected (17 animals) and were killed at 20 weeks of age for the histopathological assessment of spontaneously occurring insulitis. Four nondiabetic NOD/SCID mice were included as negative controls. Histopathology scores are shown in (C) along with representative micrographs of the NOD groups in (D). E and F, Male NOD mice were either infected on day 7 of age with *H. pylori* (8 mice) or received 3 weekly i.p. doses of 200 μg *H. pylori* extract from day 7 of age onwards (3 mice) or remained untreated (14 mice). All mice except for 4 negative controls received 1 i.p. dose of 5 mg cyclophosphamide at 10 weeks of age and were assessed histopathologically at 20 weeks of age. Histopathology scores and colony-forming units, relative to age-matched C57BL/6 mice, are shown in (E) and (F). inf., infected; extr., extract-treated; pos. ctrl., positive control; neg. ctrl., negative control.

typhlocolitis.[34] The enteroprotective effects of *H. pylori* in the latter model have been attributed to immunoregulatory sequences in the genomic DNA of *H. pylori*, which downregulate proinflammatory cytokine production and induce tolerogenic properties in dendritic cells.[35] Here, we find that the *H. pylori*–specific activation of the NLRP3 inflammasome and the subsequent processing and secretion of mature IL-18 are critical events in colitis protection. We and others have shown earlier that *H. pylori* efficiently activates the (NLRP3) inflammasome in innate immune cells to induce the autoproteolytic cleavage of caspase-1 and

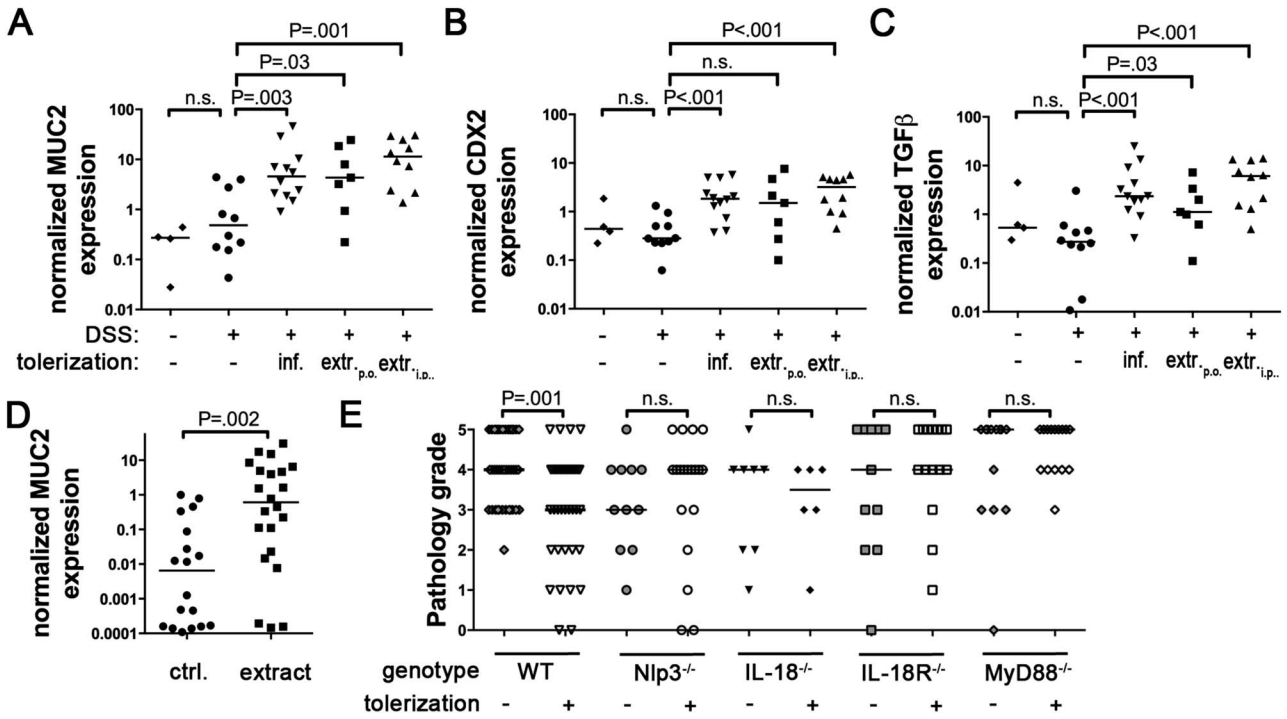

FIGURE 3. *Helicobacter pylori* infection and extract treatment induces MUC2 expression and confers protection against DSS colitis in a NLRP3-dependent and IL-18–dependent manner. A–C, C57BL/6 mice were treated as described in Figure 1. Colonic mucosal tissue was subjected to RNA extraction and qRT-PCR for MUC2, CDX2, and TGF-β transcripts; expression levels were normalized to GAPDH. D, 6-week-old mice that had been treated 3 times weekly with 200 μg *H. pylori* extract from day 7 of age onwards were subjected to MUC2 qRT-PCR, normalized to GAPDH. No DSS was administered to these mice. E, Mice of the indicated genotypes were treated with *H. pylori* extract as described in Figure 1 and exposed to 3 cycles of DSS in the drinking water. Pooled histopathology scores of 3 independent studies are shown.

the processing of pro-IL-1β and pro-IL-18 into the mature cytokines.[31,32,36] Interestingly, the 2 caspase-1 substrates fulfill very different, even opposing, functions in the *H. pylori*/host interaction. Whereas IL-1β has a strong pro-inflammatory role, promoting the differentiation of *H. pylori*–specific Th1 and Th17 cells and immune control of *H. pylori* on the one hand and infection-associated gastric immunopathology on the other,[31,32] the net effect of IL-18 signaling in the context of *H. pylori* infections is anti-inflammatory rather than pro-inflammatory. Expression of IL-18 and signaling through its receptor is dispensable for *H. pylori* infection control; in fact, mice that lack either the ligand or the receptor are capable of controlling *H. pylori* loads more efficiently because of their unrestricted *H. pylori*–specific Th17 responses.[17,31] As another consequence of excessive gastric Th17 activation, IL-18$^{-/-}$ and IL-18R$^{-/-}$ mice develop severe infection-associated immunopathology.[31] *Helicobacter pylori*–induced IL-18 is not only required for the prevention of immunopathology and for the protection against IBDs as shown here but has also emerged as a critical factor in *H. pylori*–induced asthma prevention.[17,18] In asthma, IL-18, which is secreted in large amounts by DCs that have been exposed to *H. pylori* in vitro or in vivo, promotes the differentiation of naive T cells into FoxP3^{+}CD25^{+} regulatory T cells with highly suppressive activity.[17] CD4^{+}CD25^{+} T cells isolated from the mesenteric lymph nodes of wild type, but not IL-18$^{-/-}$ and IL-18R$^{-/-}$ donors, confer asthma protection to naive recipients.[17] Whether IL-18–mediated protection in the colitis model depends similarly on the induction and function of Tregs remains to be determined. In the gut, an alternative IL-18–dependent scenario can be envisioned that involves the production of large amounts of intestinal mucins, which are known to protect against colitis.[37] Our observation that MUC2 mucus production is induced by live *H. pylori* and extract, and correlates well with protection, is reminiscent of a recently described innate immune mechanism linking the microbiota-induced activation of the NLRP6 inflammasome to goblet cell mucus hypersecretion.[37] Whereas NLRP6-dependent mucus production was attributed to mucin granule exocytosis[37] rather than transcriptional activation of MUC2 gene expression as demonstrated here, the net effect of enhanced protective mucus production is comparable in both scenarios. In conclusion, we propose here that the activation of mucus production through the NLRP3/caspase-1/IL-18 axis by *H. pylori* extract or live bacteria forms the mechanistic basis for a possible new treatment modality for IBD that is projected to be safe and cost-effective and exploits the immunomodulatory properties of a naturally occurring infectious agent known to be inversely associated with IBD risk in human populations.

REFERENCES

1. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med.* 2009; 361:2066–2078.
2. Eckburg PB, Relman DA. The role of microbes in Crohn's disease. *Clin Infect Dis.* 2007;44:256–262.
3. Rogler G. Top-down or step-up treatment in Crohn's disease?. *Dig Dis.* 2013;31:83–90.
4. Allen PB, Peyrin-Biroulet L. Moving towards disease modification in inflammatory bowel disease therapy. *Curr Opin Gastroenterol.* 2013;29:397–404.
5. Blum AM, Hang L, Setiawan T, et al. Heligmosomoides polygyrus bakeri induces tolerogenic dendritic cells that block colitis and prevent antigen-specific gut T cell responses. *J Immunol.* 2012;189:2512–2520.
6. Hang L, Setiawan T, Blum AM, et al. Heligmosomoides polygyrus infection can inhibit colitis through direct interaction with innate immunity. *J Immunol.* 2010;185:3184–3189.
7. Summers RW, Elliott DE, Urban JF Jr, et al. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology*. 2005;128:825–832.
8. Summers RW, Elliott DE, Urban JF Jr, et al. Trichuris suis therapy in Crohn's disease. *Gut*. 2005;54:87–90.
9. Dylag K, Hubalewska-Mazgaj M, Surmiak M, et al. Probiotics in the mechanism of protection against gut inflammation and therapy of gastrointestinal disorders. *Curr Pharm Des*. 2014;20:1149–1155.
10. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*. 1984;1:1311–1315.
11. Parsonnet J, Friedman GD, Vandersteen DP, et al. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med.* 1991;325:1127–1131.
12. Huang JQ, Zheng GF, Sumanac K, et al. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology*. 2003; 125:1636–1644.
13. Luther J, Dave M, Higgins PD, et al. Association between Helicobacter pylori infection and inflammatory bowel disease: a meta-analysis and systematic review of the literature. *Inflamm Bowel Dis.* 2010;16:1077–1084.
14. Wang Q, Yu C, Sun Y. The association between asthma and Helicobacter pylori: a meta-analysis. *Helicobacter*. 2013;18:41–53.
15. Sonnenberg A, Genta RM. Low prevalence of Helicobacter pylori infection among patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* 2012;35:469–476.
16. Arnold IC, Dehzad N, Reuter S, et al. Helicobacter pylori infection prevents allergic asthma in mouse models through the induction of regulatory T cells. *J Clin Invest.* 2011;121:3088–3093.
17. Oertli M, Sundquist M, Hitzler I, et al. DC-derived IL-18 drives Treg differentiation, murine Helicobacter pylori-specific immune tolerance, and asthma protection. *J Clin Invest.* 2012;122:1082–1096.
18. Engler DB, Reuter S, van Wijck Y, et al. Effective treatment of allergic airway inflammation with Helicobacter pylori immunomodulators requires BATF3-dependent dendritic cells and IL-10. *Proc Natl Acad Sci U S A.* 2014;111:11810–11815.
19. Oertli M, Noben M, Engler DB, et al. *Helicobacter pylori* g-glutamyl transpeptidase and vacuolating cytotoxin promote gastric persistence and immune tolerance Proc Natl Acad Sci U S A. 2013;110:3047–3052.
20. Arnold IC, Lee JY, Amieva MR, et al. Tolerance rather than immunity protects from Helicobacter pylori-induced gastric preneoplasia. *Gastroenterology*. 2011;140:199–209.
21. Asseman C, Mauze S, Leach MW, et al. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med.* 1999;190:995–1004.
22. Becker C, Fantini MC, Neurath MF. High resolution colonoscopy in live mice. *Nat Protoc.* 2006;1:2900–2904.
23. Codarri L, Gyulveszi G, Tosevski V, et al. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol.* 2011; 12:560–567.
24. Efrat S, Serreze D, Svetlanov A, et al. Adenovirus early region 3(E3) immunomodulatory genes decrease the incidence of autoimmune diabetes in NOD mice. *Diabetes*. 2001;50:980–984.
25. Sayi A, Kohler E, Hitzler I, et al. The CD4+ T cell-mediated IFN-gamma response to Helicobacter infection is essential for clearance and determines gastric cancer risk. *J Immunol.* 2009;182:7085–7101.
26. Powrie F, Leach MW, Mauze S, et al. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol.* 1993;5:1461–1471.
27. Allen A, Hutton DA, Pearson JP. The MUC2 gene product: a human intestinal mucin. *Int J Biochem Cell Biol.* 1998;30:797–801.
28. Yamamoto H, Bai YQ, Yuasa Y. Homeodomain protein CDX2 regulates goblet-specific MUC2 gene expression. *Biochem Biophys Res Commun.* 2003;300:813–818.
29. Jonk LJ, Itoh S, Heldin CH, et al. Identification and functional characterization of a smad binding element (SBE) in the JunB promoter that acts as a transforming growth factor-beta, activin, and bone morphogenetic protein-inducible enhancer. *J Biol Chem.* 1998;273:21145–21152.
30. Bollrath J, Powrie FM. Controlling the frontier: regulatory T-cells and intestinal homeostasis. *Semin Immunol.* 2013;25:352–357.
31. Hitzler I, Sayi A, Kohler E, et al. Caspase-1 has both proinflammatory and regulatory properties in Helicobacter infections, which are differentially mediated by its substrates IL-1beta and IL-18. *J Immunol.* 2012;188: 3594–3602.
32. Kim DJ, Park JH, Franchi L, et al. The Cag pathogenicity island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1beta production in Helicobacter pylori-infected dendritic cells. *Eur J Immunol.* 2013.
33. Wang Y, Tong J, Chang B, et al. Genetic polymorphisms in the IL-18 gene and ulcerative colitis risk: a meta-analysis. *DNA Cell Biol.* 2014;33: 438–447.
34. Higgins PD, Johnson LA, Luther J, et al. Prior Helicobacter pylori infection ameliorates Salmonella typhimurium-induced colitis: mucosal crosstalk between stomach and distal intestine. *Inflamm Bowel Dis.* 2011;17:1398–1408.
35. Luther J, Owyang SY, Takeuchi T, et al. Helicobacter pylori DNA decreases pro-inflammatory cytokine production by dendritic cells and attenuates dextran sodium sulphate-induced colitis. *Gut*. 2011;60:1479–1486.
36. Semper RP, Mejias-Luque R, Gross C, et al. Helicobacter pylori-Induced IL-1beta secretion in innate immune cells is Regulated by the NLRP3 inflammasome and requires the Cag pathogenicity island. *J Immunol.* 2014;193:3566–3576.
37. Wlodarska M, Thaiss CA, Nowarski R, et al. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014;156:1045–1059.